Could influenza be a possible biological warfare agent? Part I

Czy grypa jest możliwym czynnikiem broni biologicznej? Część I

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Abstract: Knowledge of bioterrorism in society, not only in Poland but also in the world, is scarce. A very important element in preventing and effectively counteracting the effects of biological destruction agents is to have an efficient and integrated epidemiological and virological surveillance system, as well as networks of specialized microbiological-virological accredited laboratories able to conduct rapid diagnosis. In addition, an important issue is to adequately train and equip emergency service personnel and health services acting under the developed procedures. The study in part I shows that the attacks with the use of the flu virus may have different results, from epidemic to psychological terror associated with it. The data presented are based on resources of the authors which have been done so far and on contemporary literature and oral presentation was presented at The International Conference - Advances in Pneumology. October 25-26, 2013 Kassel, Germany [1].

Streszczenie: Wiedza na temat bioterroryzmu w społeczeństwie jest niewielka nie tylko w Polsce, ale także na świecie. Bardzo ważnym elementem zapobiegania i skutecznego przeciwdziałania skutkom biologicznych środków rażenia jest posiadanie sprawnego i zintegrowanego systemu nadzoru epidemiologiczno-wirusologicznego oraz sieci wyspecjalizowanych akredytowanych laboratoriów mikrobiologiczno-wirusologicznych zdolnych do prowadzenia szybkiej diagnostyki. Ponadto bardzo ważną kwestią jest odpowiednie wyszkolenie i wyposażenie personelu służb ratowniczych i służby zdrowia działających według opracowanych procedur. W części I opracowania przedstawiono grypę w różnych ujęciach: od choroby zakaźnej po psychologiczny terror z nią związany. Przedstawione dane zostały opracowane na podstawie współczesnego dostępnego autorom piśmiennictwa oraz części ustnej prezentacji przedstawionej 25 i 26 października 2013 r. na Międzynarodowej Konferencji „Advances in Pneumology" w Kassel (Niemcy) [1]

Key words: influenza, prophylaxis, bioterrorism, biological weapon, threat
Słowa kluczowe: grypa, profilaktyka, bioterroryzm, broń biologiczna, leczenie

Introduction

Influenza viruses belong to the Orthomyxoviridae family. Vinions are spherical in shape and are surrounded by a double lipid envelope. On the basis of antigenic differences, there are three types of the influenza virus: A, B, C. Among influenza A viruses, subtypes are isolated depending on the type of hemagglutinin (HA) and neuraminidase (NA). The antigenic structure HA of the human influenza virus A contains two types of chains, as described by Wright and Webster [2]. Influenza viruses show high antigenic variability, having the nature of antigenic drift or antigenic shift. The most common mutations are observed in the case of influenza A viruses, while much less frequency is attributab-

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le to influenza B virus, whereas the influenza type C virus shows a relatively high stability. Antigenic shift consists in point mutations. The result of the accumulation of such changes in the genes which encode hemagglutinin and neuraminidase are influenza epidemics occurring every epidemic season. The antigenic shift is associated with genetic reassortment which is made possible by the segmented construction of a genome of influenza virus. This mechanism involves the exchange of entire RNA segments between different variants of viruses that infect the same cell. As a result, another variant of virus may be created with a different HA and/or NA subtype than in human strains circulating during many previous seasons, or a variant may be formed with a new subtype which has not yet occurred in humans. In this case, there may potentially arise pandemic strains, and thus capable of inducing a worldwide epidemic of high morbidity and mortality, to which the greater part of the human population will not be immune.

Flu incidences are recorded every year. Perhaps this is the reason why the flu virus is often not perceived as a threat, which is a serious mistake, especially in relation to certain groups of patients. Despite campaigns, each year in the U.S. alone ca. 40 000 Americans die due to influenza virus infection and complications from influenza in people suffering from other ailments. A large number of deaths is recorded concerning people with diseases of the lungs, heart, and kidneys, and various other conditions leading to immunosuppression. In many of these individuals the disease is prolonged to 1-2 weeks [3]. In Poland, the epidemiological surveillance of influenza is based on mandatory registration of upper respiratory tract infections, defined as incidences and influenza-like illness, and covering both respiratory infections and influenza-like illness among which influenza represents high but not fully defined percentage [4]. In Poland, according to data available on the website of the National Institute of Public Health - National Institute of Hygiene (www.pzh.gov.pl) it results that in 2013 there were 3 157 129 cases of incidences of flu and suspected cases of flu including 1 396 918 children. Deaths from influenza in the period from 1 January to 31 December 2013 were 102.

The consequences of influenza infection yesterday and today

Historical experience suggests that a greater proportion of deaths in pandemics could be caused by influenza A virus, showing the transformation of an antigen. In the most devastating pandemics in 1918-1919 (Spanish flu) died between 20 to 40 million people [5]. A large percentage of deaths occurred in a group of young people in the age of 15-35, who did not undergo other illnesses. However, since the influenza virus, until 1933, was isolated from patients [6], the strain of this virus from pandemic of 1918-1919 [6] was not preserved. However, nowadays through the analysis of the preserved samples from the autopsy it is possible to determine the sequence of certain portions of the genomic RNA [5]. However, the recovered sequences do not allow yet to explain the reasons for the extraordinary infectiousness and severity of the disease. One hypothesis is that the population of patients aged 15-35, as opposed to the older population, has never been exposed to the virus strains having antigenic similarity to the pandemic virus and consequently the natural immune system in this age group was not able to respond.

We also have a very contemporary experience of an influenza epidemic in Hong Kong in 1997. There is no doubt as to the high virulence of the virus isolated there [8-10]. Influenza virus that has been brought directly from chickens to humans infected 18 people, of which as many as 6 died. This strain of the virus contains an HA gene segment (H5HA) from virus of goose influenza (A/Goose/guantang/1/96) and seven gene segments of influenza virus of teal (A/Teal/HK/1/97) [2]. Because this strain also contains an N1 neuraminidase gene (NA), it has been classified as an H5N1 virus. Into the virulence of this strain were mixed in specific sequences at least in the last two encoded proteins (HA and PB2 subunit of viral polymerase) [11]. Fortunately, there is no transmission of this virus from person to person and the acquisition of this property requires a mutation and/or mixing the genes with the virus of human influenza. It was believed that in Hong Kong this phenomenon was prevented by a quick killing and disposal of dead poultry [2]. Since a similar type A virus still circulates in Asian shopping malls [12], it can again be transferred from chickens to humans. At some point, it may be impossible to prevent an epidemic because of the acquisition by the virus the ability to transmit from human to human. In other words, the emergence of new virion particles capable of rapid spread can be a great challenge and will require a strategy similar to that which is taken at preventing a bioterrorist attack because such naturally occurring highly pathogenic virus can be used as a biological weapon.

Flu and bioterrorism

It is likely that the deadly human influenza A virus can be produced in laboratories by repeated

DNA transfection using the genetic reversion system [13, 14]. It has been even reported that by using this system, a pathogenic H1N1 virus was produced [11, 15]. It is believed that the same DNA recombinant technology may be applicable for transmitting the virus A/H1N1 from person to person. Moreover, it is likely that such a mutation will be introduced to make it resistant to current antiviral inhibitors (M2 inhibitor: amantadine and rimantadine, and an NA inhibitor: zanamivir and oseltamivir) [16, 17].

As a consequence, the human population would not be resistant against such viruses and the existing antiviral drugs would not provide any protection. For terrorists it would be a perfect biological weapon, considering that such viruses will be shed secretly in populated areas by simple equipment producing aerosols.

Fortunately terrorists, as yet, do not have knowledge of genetic engineering and equipment to conduct experiments with DNA recombination. This is probably at the moment, but in the future the situation may unexpectedly change. It should be taken into account that the terrorists will not be deterred by the risks associated with the production of lethal strains of viruses, because they are groups determined to sacrifice life for their purposes. Examples of this are the terrorists, who made attacks on the WTC in New York in 2001.

Preventing possible cases of deliberate infection with influenza virus

It will be prudent to maintain the lead in the preparation of the countermeasures against the use of influenza virus as a weapon of terror.

And so, in the area of specific prevention, mass vaccinations are likely to have limited significance. Currently, the preparation of a vaccine against the new strain of influenza virus takes about six months. Perhaps the achievements of genetic reversion can shorten this time, but, nevertheless, there will be a gap in time between the outbreak of the influenza epidemic and the availability of a protective vaccine. In addition, vaccine protection may be thwarted by bioterrorists who would have highly pathogenic strains of the influenza virus.

A more effective protection can be expected in the area of chemotherapeutic prevention. Drugs that may be used for the prophylactic prevention of influenza virus infection type A, will be the best defence against a terrorist attack. Currently, the only available antiviral drugs are NA inhibitors (zanamivir and oseltamivir) [17]. It would therefore be sensible to maintain strategic reserves of inhibitors and possibly other antiviral drugs. Consequently, in such proceedings it would be advisable, if possible, to organize a defensive stocks in the event of a natural or terrorist spread of the H1N1 virus transmitted from human to human.

With the adoption of such a defence doctrine there is the need for introduction of further intensive research on new generations of antiviral drugs. One of them was a promising viral RNA polymerase. In the past, a number of investigators were working on compounds, that specifically inhibit the enzyme activity, based on the premise that it triggers a response unique to viruses. These inhibitors have not yet been tested on humans and animals [26, 27], but recent studies [18] have questioned the possibility of their use, as they actively destroy polynucleotides up to the formation of toxic chain ends at the end of the 3'-OH. As a result, the toxicity of this type of antiviral drugs may be not acceptable.

Other viral proteins also arouse interest. For example, proteins involved in the penetration of the virus into the cell through the intermediary of the HA [19] seem to be interesting. Another interesting protein is the non-structural viral protein (NS1), in particular its interaction with two cellular proteins that are involved in the production of cell pre-mRNAs [20–23].

The works on the new antiviral agents may be carried out on the basis of multiple strains of influenza A virus, which many different laboratories have. Consequently, these works may also be carried out in any laboratory that meets the conditions for research on virus A/H1N1 (BSL-3).

The application of an effective chemotherapeutic prevention will require compliance with the following procedure:

1. The isolation of a newly disclosed lethal strain(s) of influenza virus will be necessary.
2. The distinction of the obtained isolate from other pathogens that cause similar symptoms.
3. Although currently there are sets of distinguishing pathogens, showing the nature of viruses, from bacteria, but there is also a need for sensitive tests that will define clearly and fast, if there are in the population new mutant strains of influenza A virus.

As soon as a bioterrorist spreads influenza A virus, after the diagnosis, antiviral drugs should be administered. Because influenza A virus is highly contagious, antiviral drugs should be administered not only to persons showing signs of an illness or those in contact with them, but also to people from their close

and distant environment. Hence, it is important to emphasize the importance of having a stock of antiviral drugs for influenza cases of epidemic threats.

Even with a specific, coordinated effort, developing new, safe, effective antiviral drugs is a long-term project, one of those that will probably take at least 5–10 years. Thus, there is already a need for the start of this program in order to gain an advantage in time before the flu virus appears that will be available for bioterrorists as a weapon. Of course, emphasis should be given to make the results of these efforts also necessary to combat future naturally widespread epidemic of influenza A.

Political aspects of combating threats of the flu

Problems concerning preventing and combating bioterrorist threat of the use of influenza virus are also not solved on the political level.

The changing approaches of consecutive U.S. administrations, Bush and Obama, are the object of a detailed study carried out by the Koblenz, entitled *From bio-defence to biosafety* [24]. During the period of the attempts to implement the decisions of the Convention on the Prohibition of Biological Weapons (BWC) in the environments of biologists there was a discussion about the possibility of a dual-use of biological sciences prey for peaceful purposes, but also for the construction of biological weapons.

Even with regard to avian influenza virus A/H5N1/ there was a ban on publishing research on the genetic alteration. National Science Advisory Board for Bio-safety presented its point of view in that case [25]. The advisers pointed to the threat of scientific freedom posed by the censorship. In the cases of this research, referring to the freedom of research are sufficient to support the rejection of censoring.

In May 2009, President Obama announced the release in 6 years $ 63 billion for the Global Health Initiative, which means shifting resources from the defence against biological weapons threats to developing medical projects on pandemic and emerging new infectious diseases.

The focal point of the Obama administration's approach is to strengthen the international disease surveillance and to respond on the basis of the international regulations of the WHO from 2005 (WHO International Health Regulations [IHR]).

The first example of this approach to balance science and bio-security is the policy of the Obama administration for the prevention of improper use of synthetic genomics. Synthetic Genomics enables scientists to construct synthetic-based viruses on the basis of a long chain of nucleotides – building blocks of DNA – obtained from commercial suppliers. Since synthesizing the first virus in 2002, there is a growing interest that this technology could allow terrorists to receive threatening pathogens in spite of laboratory biosafety and elimination of such pathogens in nature. Gene synthesis industry, made up of a dozens of private companies in the U.S., Europe and China engaged in it individual resources to develop safety devices against misuse, but no industry standard appeared.

The change of this strategy began in December 2009, when the Obama administration announced a major political initiative, called the National Strategy for Countering Biological Threats (NSCBT).

This strategy involves changing the proceedings: from focusing on the defence, which was promoted by the Bush administration, on the prevention and response against both naturally occurring and deliberately initiated biological threats.

The main objective of this strategy is to reduce the risks resulting from misuse of the achievements of biology, not admitting to intentional or unintentional sharing of biological material for causing mass sickness or death of the population, for causing death of animals and withering of crops.

To achieve these goals the key are: prevention, international cooperation and a causal relationship between health and safety. In addition, the Obama administration's strategy highlights the risks for the entire world community posed by biological threats, while the Bush administration assumed biological weapons primarily as a threat to the United States and its allies.

The opinion of the Bush administration on global health was dominated by the major initiatives focusing on HIV/AIDS and the response to influenza: avian and human pandemic influenza. Of $ 60 billion spent on bio-defence of the U.S. in 2001-2009, only 2% (1.1 billion) has been spent on preventive measures, such as national bio-security laboratories, export controls and biological threat reduction programs abroad. Over 40% (26.3 billion) has been spent on research and development of new medical countermeasures, diagnosticians, sensors and techniques to eliminate the consequences of the terrorist use of biological agents.

For these reasons, today life sciences and their infrastructure is perceived as the centre of preventive actions. Laboratories with high bio-safety of type III and IV are key elements as well as the protection of the collection of pathogens that can be used as a terrorist weapon. Given the high level of *know-how* needed in

order to use disease as a weapon of mass destruction, the essence of the doctrine of the Obama administration is the statement that "less attention should be focused on the fact that terrorists do not become biologists and more on the fact that biologists do not become terrorists". For the assessment of the risks that can arise from biological research, the Obama administration will develop detailed guidelines. The need for such guidelines was shown by controversies that emerged around the possibility of transmission of H5N1 influenza virus from birds carriers to humans, which occurred in Asia in 2005 and the pandemic of human influenza caused by the same type of virus in 2009.

In October 2010, the U.S. Department of Health published a guide, which describes the main recommendations to make the owners of companies producing custom DNA sequences register and subsequently checked for the activity not compliant with registered specifications. If the inspection reveals any suspicions, the supplier of genes is encouraged to contact the FBI and other relevant government agencies. Meeting the requirements of such guidelines is voluntary at this stage, but the important fact is that these guidelines determine the scope for providing responsible conduct and identifying the channels of communication in case of detection of any ambiguity. Although the gene synthesis industry and scientists have many objections to this guide, the American Association for the Advancement of Science states, however, that "the general feeling is that the well thought guide facilitates progress in science and allows for precise determination of safe international obligations".

The true measure of success of the strategy of the Obama administration will be the introduction of a road map for future politicians, when they will settle the problems of double use of the results of biological research, link the activities of health and safety services in strengthening health security in a global sense. Politicians and scientists from European countries should also take into account similar solutions in the near future.

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